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Reports of the ACMRR/IABO Working Party on

BIOLOGICAL EFFECTS OF POLLUTANTS

First Session, Rome, Italy, 27-31 October 1975
Second Session, Dubrovnik, Yugoslavia, 22-25 November 1976

and the ACMRR/IABO Expert Consultation on

BIOASSAYS WITH AQUATIC ORGANISMS IN RELATION TO POLLUTION PROBLEMS

Dubrovnik, Yugoslavia, 16-19 November 1976

with the cooperation of the
United Nations Environment Programme

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

FAO Fisheries Report No. 187
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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

UNITED NATIONS ENVIRONMENT PROGRAMME

REPORT OF THE ACMRR/IABO WORKING PARTY ON BIOLOGICAL EFFECTS OF POLLUTANTS

First Session
FAO Headquarters
Rome, Italy, 27-31 October 1975

Second Session
Inter-University Centre for Post-graduate Studies
Dubrovnik, Yugoslavia, 22-25 November 1976

and

REPORT OF THE ACMRR/IABO EXPERT CONSULTATION ON BIOASSAYS WITH AQUATIC ORGANISMS IN RELATION TO POLLUTION PROBLEMS

Inter-University Centre for Post-graduate Studies
Dubrovnik, Yugoslavia, 16-19 November 1976

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 1977

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PREPARATION OF THIS REPORT

This report was prepared as part of a cooperative project of the United Nations Environment Programme entitled

> Effects of Pollution on Living Aquatic Resources and Scientific Basis for Monitoring

with the Food and Agriculture Organization of the United Nations as cooperating agency.

Travel funds for experts attending the ACMRR/IABO Expert Consultation on Bioassays with Aquatic Organisms in Relation to Pollution Problems were made available from the above-mentioned project, the Joint FAO(GFCM)/UNEP Coordinated Project on Pollution in the Mediterranean and the Swedish International Development Authority (SIDA).

As a result of the Working Party on Biological Effects of Pollutants, the following publication is in preparation and will be published as FAO Fisheries Technical Paper No. 164:

> Manual of Methods in Aquatic Environment Research
> Part 4. Bases for selecting biological tests to evaluate marine pollution

Distribution

FAO Department of Fisheries
FAO Regional Fishery Officers
Selector SM
ACMRR Members
UNEP

Bibliographic reference

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Water pollution. Bioassays. Toxicity tests. Water quality. Pollution monitoring.

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REPORT OF THE FIRST SESSION OF THE ACMRR/IABO WORKING PARTY ON BIOLOGICAL EFFECTS OF POLLUTANTS

Rome, Italy, 27-31 October 1975

1. OPENING OF THE SESSION

The First Session of the ACMRR/IABO Working Party on Biological Effects of Pollutants was held at FAO Headquarters, Rome, from 27 to 31 October 1975, under the chairmanship of Dr. J.B. Sprague. Dr. H. Kasahara, Director, Fishery Resources and Environment Division, welcomed the members (listed in Annex III), and opened the session on 27 October.

2. ADOPTION OF THE AGENDA

The Agenda (Annex I) was adopted without changes.

3. ELECTION OF RAPPORTEUR

Dr. P.A. Butler was elected Rapporteur of the session.

4. DISCUSSION OF TERMS OF REFERENCE

The meeting opened with a general discussion on the importance of the different approaches to the identification and study of pollution in marine environments. It was recognized that substantial contributions had been published on the methodology of toxicity testing and determination of water quality criteria in the freshwater environment, and that some of the basic approaches were applicable to the identification and management of similar problems in the marine environment. It was further recognized that there are several groups currently working on assessments of testing procedures for evaluating marine pollution. It was agreed that, within the terms of reference, the Working Party should concern itself especially with the rationale for the use of toxicity tests and the different approaches to marine pollution problems rather than with the details of test protocols which are available elsewhere.

There was discussion about directing the report to those who would find it most useful and it was agreed to include material that would assist in the management of fisheries and in the protection of the marine environment, especially in those areas where pollution was an incipient problem or where trained personnel were only now becoming available to work on pollution problems.

The different methods of bioevaluation, including both acute and chronic tests, to identify specific pollutants were explored, as well as the very short-term tests which are used primarily to identify stress conditions rather than individual pollutants. It was decided that a brief tabulation of the most important purposes of each of the many kinds of bioevaluation described in the literature would be helpful and should be included in the report.

It was agreed that, in the context of studying pollution problems and identifying the toxicity of specific substances or elements in the environment, the selection of the proper test organisms was essential to an understanding of the problems. Therefore, the report will include a brief tabulation of the kinds of organisms that have been used under different conditions, the use of standard strains and cultures, and will provide pertinent references to the successful use of the different taxa of test organisms.

There was general agreement that, as an additional topic, a discussion of the purpose and use of pollution loading rates would be useful in relating laboratory test data to the management of estuarine and coastal zone resources.

5. OUTLINE OF FINAL REPORT

An outline of the technical report was prepared. During the meeting the group completed a first draft of the report based on this outline.

6. FUTURE WORK

The Working Party decided to polish the draft report, send it for comments to technical experts and expand it as seems necessary in view of comments received. This work is to be completed by early or mid-1976. The second meeting is tentatively to be held during the first half of October 1976 when the report will be finalized.

7. PREPARATION FOR AN EXPERT CONSULTATION

The Working Party, at the request of the secretariat, discussed the need for and the possible objectives of an expert consultation to be held in conjunction with the second meeting. It was agreed that such an expert consultation would be useful to cover current and impending marine pollution problems and to assist in orientation of the report. Topics for the expert consultation were discussed and a draft outline for a list of topics was suggested to the secretariat. Such an expert consultation could be a useful meeting ground for experts and management personnel who were undertaking new pollution investigations. The expert consultation should be held in conjunction with and immediately preceding the group's second meeting. It was left to the secretariat to decide on the place for the expert consultation.

8. ANY OTHER MATTERS

The Working Party, at the request of the secretariat, reviewed some of the lectures presented at the current Fourth FAO/SIDA Training Course on Aquatic Pollution in Relation to Protection of Living Resources dealing with bioassay and toxicity testing. It was felt that after completion of all the lectures a selection of them should be published after thorough editing by an expert, perhaps as a contract effort.

9. ADOPTION OF THE REPORT AND CLOSING OF THE SESSION

The Report was adopted on 31 October 1975. Editorial changes were left to the secretariat.

Mr. Wenblad thanked the members of the Working Party for their work and the meeting closed on 31 October 1975.

REPORT OF THE ACMRR/IABO EXPERT CONSULTATION ON BIOASSAYS WITH AQUATIC ORGANISMS IN RELATION TO POLLUTION PROBLEMS

Dubrovnik, Yugoslavia, 16-19 November 1976

1. OPENING OF THE SESSION

The ACMRR/IABO Expert Consultation on Bioassays with Aquatic Organisms in Relation to Pollution Problems was held at the Inter-University Centre for Post-graduate Studies, Dubrovnik, from 16 to 19 November 1976, under the chairmanship of Dr. D.J. Reish. The participants (listed in Annex IV) were welcomed by Dr. P. Strohal, on behalf of the Host Country, and by Dr. H. Naeve, on behalf of FAO.

2. ADOPTION OF THE AGENDA AND ELECTION OF RAPPORTEUR

The Agenda (Annex I) was adopted without change.

Dr. J.V. Bannister was elected Rapporteur of the Session.

3. DRAFT REPORT OF THE ACMRR/IABO WORKING PARTY ON BIOLOGICAL EFFECTS OF POLLUTANTS

Document FI:ACMRR/BE/4, Bases for Selecting Biological Tests to Evaluate Marine Pollution, was presented by Dr. P.A. Butler. He requested from the participants critical assessment as to a review of the sources of pollution, methods of testing and type of organisms to be tested. Mr. R. Lloyd described the various methods of testing and emphasized the importance of knowing the chemical nature of the substance tested. The flow-through method of bioassay was considered to be the best, but static bioassay is especially useful in a screening-type test. Dr. M. Swedmark indicated that, in using a test organism, a study of its biology is essential and that the animal should survive without difficulty in the laboratory. The selection of the test organism was discussed by many of the participants. It was decided that the choice of the test organisms should depend on what is being tested. Dr. P. Doudoroff presented the interpretation of the results and the application of the results and the toxicity emission rate equation. Variations in the results were explained in terms of growth, reproduction, locomotion and food availability. He also stated that appropriate application factors can be different for different environments, because different levels of protection of aquatic life may be needed for social and economic reasons. It was suggested that application factors are more applicable to effluents than to individual chemicals.

The recommendations of the Consultation on the report were:

(i) to expand reasons for selecting test organisms;

(ii) to give more attention to chemical aspects of bioassay procedures;

(iii) to further clarify Section 7, on specific uses of different tests, with more emphasis on the practical application of the tests;

(iv) to provide an appendix, giving examples of the usage of the report in practice;

(v) to clarify Appendix III, dealing with the toxicity emission rate;

(vi) to include examples at relevant stages in the Report to improve the clarity of the presentations.

4. EXPERIENCE AND PROBLEMS WITH BIOASSAY TECHNIQUES

A list of papers presented by the various participants is given in Annex V, and a summary of each paper is given in Annex VI. Each paper was followed by a short discussion, and Dr. P.A. Butler presented some comments on these papers. He considered that the data were very well presented, that the experience is there, but that there is much more work to be done in order to solve particular problems. A greater use of the scientific literature and comparisons of results with previous investigations in other countries were suggested.

5. ASSESSMENT OF POLLUTION EFFECTS BY CHEMICAL, TOXICOLOGICAL AND OTHER BIOLOGICAL METHODS

Dr. D. Calamari presented Documents FI:ACMRR/BE/12, 13 and 14, prepared in the framework of the activities of Subcommission III of the European Inland Fisheries Advisory Commission (EIFAC), on the value and limitation of various methods for the assessment of water quality criteria for fisheries. He discussed how the various monitoring information can be utilized, and informed the Consultation that the final document will be available as an EIFAC document in due course.

6. ASSISTANCE TO DEVELOPING COUNTRIES

Dr. D.J. Reish presented a paper on the possible means of assistance to developing countries concerning aquatic pollution problems as related to fisheries. He discussed training programmes of various types, establishment of scientific advisory groups and problems of availability of scientific literature. During the discussion, the following recommendations were made:

(i) Training courses on aquatic pollution should be continued to be organized by FAO, bearing in mind the needs of developing countries.

(ii) Accessibility of scientific literature was considered important and therefore FAO should facilitate access to existing literature.

(iii) Links between a university or a governmental laboratory of a developing country and that of a developed country were considered desirable, thus furthering research programmes and training.

(iv) The establishment of a scientific advisory mechanism, coordinated by FAO, was considered useful to give, through correspondance, advice on practical problems related to aquatic pollution, if and when requested by individual scientists in developing countries.

(v) FAO should continue to provide technical assistance in obtaining the necessary scientific equipment and providing for the training of technicians in the use and maintenance of the equipment.

(vi) Further meetings of the type of this Consultation should, from time to to time, be held, preferably in developing countries where related studies are being carried out, since the present meeting provided a good opportunity for scientists from developing and developed countries to meet, discuss mutual problems, exchange information and ideas.

7. DEVELOPMENT OF WATER QUALITY CRITERIA

Mr. R. Lloyd presented Document FI:ACMRR/BE/20 on the work of Subcommission III of the European Inland Fisheries Advisory Commission (EIFAC) to establish Water Quality Criteria for European Freshwater Fish. As an example of this work, the Report on Zinc and Freshwater Fish was presented (Doc. FI:ACMRR/BE/16).

The group appreciated the work done by EIFAC and discussed in depth problems and limitations in the establishment of water quality criteria. Special reference was made to the discussions held under Agenda Item 4, "Experience and Problems with Bioassay Techniques".

Dr. H. Naeve introduced the Report of the GESAMP Working Party on Principles for Developing Water Quality Criteria (Doc. FI:ACMRR/BE/15).

During the discussions, special attention was given to the fact that, according to the document, a lot of information is required for establishing coastal water quality criteria. It was proposed to define the minimum information required to establish provisional criteria which later, on the basis of additional information, could be revised. Reports of this kind would be welcomed as a help in establishing water quality criteria for scientific and legal purposes.

8. ADOPTION OF THE REPORT AND CLOSING OF THE SESSION

The Report was adopted on 19 November 1976.

Dr. H. Naeve thanked Dr. D.J. Reish for chairing the Consultation and asked Dr. P. Strohal to receive, on behalf of the Host Country, the appreciation of the meeting for the excellent technical arrangements.

The meeting was closed on 19 November 1976.

REPORT OF THE SECOND SESSION OF THE
ACMRR/IABO WORKING PARTY ON
BIOLOGICAL EFFECTS OF POLLUTANTS

Dubrovnik, Yugoslavia, 22-25 November 1976

1. OPENING OF THE SESSION

The Second Session of the ACMRR/IABO Working Party on Biological Effects of Pollutants was held at the Inter-University Centre for Post-graduate Studies, Dubrovnik, Yugoslavia, from 22 to 25 November 1976. In the absence of Dr. J.B. Sprague, the chair was taken by Dr. P.A. Butler. Mr. R. Lloyd was nominated as Rapporteur for the meeting. The meeting was opened by Dr. B. Kurelec, Center for Marine Research, Rovinj, Yugoslavia, who welcomed the Working Party members and FAO Secretariat (listed in Annex III).

2. ADOPTION OF THE AGENDA

The Agenda (Annex I) was adopted without changes.

3. REPORT OF THE ACMRR/IABO EXPERT CONSULTATION ON BIOASSAYS WITH AQUATIC ORGANISMS IN RELATION TO POLLUTION PROBLEMS

This Report contained six recommendations from the Expert Consultation for revision and expansion of the Draft Report of the Working Party to improve its clarity and usefulness. These recommendations were:

(i) to expand reasons for selecting test organisms;

(ii) to give more attention to chemical aspects of bioassay procedures;

(iii) to further clarify Section 7, on specific uses of different tests, with more emphasis on the practical application of the tests;

(iv) to provide an appendix, giving examples of the usage of the report in practice;

(v) to clarify Appendix III, dealing with the toxicity emission rate;

(vi) to include examples at relevant stages in the Report to improve the clarity of the presentations.

The Working Party welcomed these recommendations and agreed to attempt the appropriate revision of the Draft Report.

4. REVISION OF THE DOCUMENT "BASES FOR SELECTING BIOLOGICAL TESTS TO EVALUATE MARINE POLLUTION" (FI:ACMRR/WP/BE/76/2)

The main task of the Working Party during the meeting was to revise the draft document and prepare the additional information as recommended by the Expert Consultation. This was completed and the Report was finalized for editing and publication.

The following recommendations were made:

The Working Party recommends to FAO that:

(i) The Report "Bases for Selecting Biological Tests to Evaluate Marine Pollution" should be published by FAO; that it should contain a list of Working Party Members who had contributed to the preparation of the Report; and that FAO should provide an appropriate "Foreword" to the

Report. The draft contents table of this publication is given as Annex VII.

The Working Party further recommends to FAO that:

(ii) Because of the considerable value of the Expert Consultation in assisting the Working Party members in their task, as well as the assistance Working Party members were able to give to scientists from developing countries, similar expert consultations should be held in conjunction with the meetings of other similar technical working parties set up by FAO and its subsidiary bodies.

(iii) The recommendation listed in the Report of the Expert Consultation (see page 4) as items 6(i)-(vi) should be acted upon, with priority being given to recommendations (i), (iv) and (vi).

(iv) Further to the Report "Bases for Selecting Biological Tests to Evaluate Marine Pollution", and in fulfillment of item (iii) of their terms of reference, a consultant should be appointed to compile a manual of bioassay and bioevaluation techniques which have been published in standard works and in the scientific literature. The consultant should be assisted by a panel of experts. The techniques chosen should be critically assessed and their value, advantages and shortcomings listed in the manual. This manual should be widely distributed, especially to developing countries.

5. ADOPTION OF THE REPORT AND CLOSING OF THE SESSION

This Report was adopted by the Working Party on 25 November 1976.

The Chairman of the Session paid tribute to the invaluable assistance provided by Dr. B. Kurelec and the Secretariat in helping the Working Party in their task. He thanked all those members of the Working Party, and others present, who had contributed to the preparation of the Report, and closed the meeting on 25 November 1976.

Annex I

AGENDAE

Agenda of the
First Session of the ACMRR/IABO Working Party
on Biological Effects of Pollutants

1. Opening of the Session
2. Adoption of the Agenda
3. Election of Rapporteur
4. Review of Terms of Reference
5. Outline of Final Report
   - 5.1 Rationale
   - 5.2 Types of tests
   - 5.3 Test procedure
   - 5.4 Additional topics
6. Future Work
7. Preparation for an Expert Consultation
8. Any Other Matters
9. Adoption of Report and Closing of the Session

Agenda of the
ACMRR/IABO Expert Consultation
on Bioassays with Aquatic Organisms
in Relation to Pollution Problems

1. Opening of the Session
2. Adoption of the Agenda
3. Draft Report of the ACMRR/IABO Working Party on Biological Effects of Pollutants
4. Experience and Problems with Bioassay Techniques
5. Assessment of Pollution Effects by Chemical, Toxicological and Biological Methods
6. Assistance to Developing Countries
7. Development of Water Quality Criteria
8. Adoption of the Report and Closing of the Session

Agenda of the
Second Session of the ACMRR/IABO Working Party
on Biological Effects of Pollutants

1. Opening of the Session
2. Adoption of the Agenda
3. Report of the ACMRR/IABO Expert Consultation on Bioassays with Aquatic Organisms in Relation to Pollution Problems
4. Revision of the Document "Bases for Selecting Biological Tests to Evaluate Marine Pollution"
5. Adoption of the Report and Closing of the Session

Annex II

TERMS OF REFERENCE
ACMRR/IABO Working Party
on Biological Effects of Pollutants

As a basis for decision-making on management of the aquatic environment and specifically on the regulation of waste disposal, with the aim of protecting the living aquatic resources and fisheries and establishing selected monitoring programmes:

(i) to review the present use of methods on bioevaluation, including toxicity tests to measure short- and long-term effects of pollution on selected aquatic species;

(ii) to evaluate critically the methods used in bioassay and toxicity tests to assess their accuracy and reliability with special regard to methods of obtaining test organisms, and to advise on their value for control and research purposes and for general use in environmentally different geographic areas;

(iii) to select working methods and to prepare detailed proposals for their intercalibration and eventual standardization with regard to mortality and survival rates, chronic and sublethal effects on biological processes and activities (e.g. growth, behaviour, reproduction, etc.) of various species and for various pollutants, taking into account also bioaccumulation processes and the problems of joint toxicity;

(iv) to advise on the practical use of tests to improve the use of data from laboratory tests, field experiments and field observations, including the possible use of organisms and tissues under culture;

(v) to identify research programmes required to improve methodology and their application to the establishment of criteria and standards (e.g. water quality, tolerance limits, etc.)

Annex III

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ACMRR/IABO Working Party
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Annex V

LIST OF DOCUMENTS

ACMRR/IABO Expert Consultation
on Bioassays with Aquatic Organisms
in Relation to Pollution Problems

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Annex VI

ABSTRACTS OF PAPERS PRESENTED AT THE ACMRR/IABO EXPERT CONSULTATION ON BIOASSAYS WITH AQUATIC ORGANISMS IN RELATION TO POLLUTION PROBLEMS

SOME PROBLEMS IN BIOTESTING IN THE PHILIPPINES

by

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Acute toxicity tests of heavy metals, pesticides, thermal and industrial effluents on economically valuable fish species, Chanos chanos (milkfish), Cyprinus carpio (common carp) and Tilapia mossambica (cichlid), are carried out in the Water Research Laboratory of the National Pollution Control Commission. The biotests aim at providing results applicable towards the establishment of ecologically meaningful criteria to improve existing national standards that were previously promulgated by legislation.

At present, only the interaction between a fish and a pollutant under laboratory conditions is studied. Ecological studies will be started in the near future. Research on the histochemical and enzymatic level has not been done due to lack of technical know-how and proper equipment.

One pressing problem encountered during the course of biotesting is difficulty in acclimating test fishes, especially the milk fish. Wide variations in 96 h LC50 values for different test species indicate problems on how to set up realistic criteria for fish. The identification and ranking of toxic components in complex industrial effluents and the provisions for synergism and antagonism also pose certain problems.

A question of priority arises in the application of biotest results. Are effluent standards more feasible than criteria for aquatic life and water quality?

Algal assays might prove useful in discovering the trigger mechanism and predicting the occurrence of algal blooms in tropical waters. Since not much literature exists on the subject, guidance from experts is necessary.

Very few laboratories carry out pollution research in the Third World. There is, therefore, an urgent need for exchange of data and information. Concrete efforts should also be exerted to initiate intercalibration because improvement, development and application of methods depends on it.

BIOCHEMICAL STUDIES ON POLLUTION PROBLEMS*

by

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The programme of research that is being carried out concerns the physiological and biochemical responses of marine species to pollution by pesticides. These investigations will be concerned with the measurement of the activity levels of key metabolic enzymes in the liver and muscle over long exposure periods at sublethal concentrations. The emzyme activities to be measured are lactate dehydrogenase, pyrurate kinase, malate dehydrogenase, succinate dehydrogenase and cytochrome oxidase. Another type of investigation concerns the measurement of changes in the blood composition during exposure. Tests will be made to discover any haematological changes and changes in the ionic composition.

Preliminary investigations have been carried out to test the *in vitro* effects of pesticides on enzymes. Such investigations have so far served the purpose of giving information about the handling of species, choice of tissue and methods of assay. *In vivo* exposures, coupled with the physiological and biochemical investigation, are now being initiated. Such investigations should provide a better understanding of the mode of action of pesticides on metabolic pathways and eventually to permit analyses to be carried out on natural fish populations.

TOXICITY TEST USING ATP (ADENOSIN TRIPHOSPHATE) AS PARAMETER*

by

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ATP is a biochemical component present in all live organisms at an almost constant level. This compound is very labile in the organism and is broken down whenever energy is released, and built up again by energy supplied through the respiratory chain, as well as by photosynthetic activity. When death of the organism occurs, ATP is immediately cleaved by the phosphatase to ADP and inorganic phosphate. The determination of ATP can be used as an indicator of toxic effects.

In the laboratory of the Israel Oceanographic and Limnological Research Institute experiments have been initiated with several oil dispersants. The table below summarizes the findings on the effects of three oil dispersants on the ATP content in a sample of lake water, after exposure of this water for two hours to the dispersants. As can be seen, there are differences in sensitivity of micro-organisms inhabiting the water sample to the different dispersants.

This bioassay test should not substitute regular bioassays using fish and other aquatic organisms as test organisms; however, it might be used as fast-screening test.

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*Not presented as meeting document

Dispersant concentration ppm	ATP Concentrations (µg/l)		
	88 oil slick dispersant	TS-5	Polycomplex A
0	0.132	0.132	0.132
65	0.150	0.144	0.027
650	0.084	0.036	0.018
6500	0.091	0.018	0.022

DETERMINATION OF TOXICITY LEVELS OF CERTAIN PESTICIDES IN INDIGENOUS FISH SPECIES IN COLOMBIA

by

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Since Colombia's economy is agriculture-oriented, there exist about 250 pesticide formulations which are sprayed, either aerially or manually, for pest control purposes. Control regulations as to their application are mainly concerned with handling over basins, wind direction, flight height and distance from the water line. However, nothing is known of their concentrations in relation to noxious effects on aquatic biota.

Within the framework of the recently established Codex of Natural Resources and Environment, the technique of bioassays has been introduced, based on the Tarzwell postulate "all or nothing", the contaminant kills or does not kill.

Due to the wide range of pesticides used, primary pesticides (organochlorines) such as Chlordane, Heptachlor, Toxaphene, Aldrin and Endrin, of a 99.9% field grade purity, were selected for their wide application in the medium.

The species examined were Salmo gairdnerii, Petenia umbrifera, Lebistes reticulatus, Pimelodus clarias and Prochilodus reticulatus magdalenae. The criteria followed in the selection were:

1. Standardization of results
   Salmo gairdnerii and Lebistes reticulatus
2. Species resistance, with the aim of assessing tolerance, taking into account that Petenia umbrifera is the most resistent species and that Salmo gairdnerii is the most sensitive
3. Commercial importance as local food
   Prochilodus reticulatus magdalenae and Pimelodus clarias
4. Different trophic levels

A screening test is then carried out for the preliminary bioassay as it permits the determination of threshold values for pesticides on test animals; once the data are obtained, a continuous-flow toxicity test is carried out.

FISH HEALTH EXAMINATION FOR POLLUTION EFFECTS ON AQUACULTURE

by

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As emphasized during the FAO Technical Conference on Aquaculture in Kyoto, Japan, in 1976, aquaculture will be of primary importance to the future development of world fisheries. Therefore, the future system of water pollution control should be established with the inclusion of aquaculture.

The essence and significance of aquaculture and the effects of pollutants on aquaculture (in terms of seed production, release and growth) are explained. Also, the principle and the problems of accumulation of toxicants are discussed.

Fish health examinations, with morphological, haematological, biochemical, serological and histopathological techniques, are outlined, and the importance of future pollution control is emphasized. Some test procedures for pollution control in aquaculture are proposed.

Sites suitable for aquaculture along the coastal areas are also claimed for other purposes such as industrialization, urbanization and recreation. It is therefore suggested to collect strong scientific information in support of the protection of sites for aquaculture development.

SOME OBSERVATIONS ON THE POLLUTION OF THE HOOGHLY ESTUARY IN THE VICINITY OF A RAYON INDUSTRY WITH A NOTE ON THE TOXICITY OF THE EFFLUENT

by

B.B. GHOSH, P. RAY, D.D. HALDER and M.M. BAGCHI
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An account of the investigations carried out during 1970-73 on the pollution status of the Hooghly Estuary in the vicinity of a Rayon industrial complex discharging toxic metals such as zinc in acidic condition, along with a suggestive measure of dilution or treatment necessary, based on bioassay toxicity experiments, for the healthy survival of plankton populations and a commercially important shrimp fishery, has been presented. Process-wise characterization of the waste waters, seasonal characteristics of the combined effluent discharged into the estuary and their impact on the hydro-biological conditions of the estuary during the dry-weather period (November-June) have been indicated. During summer (March-June) the plankton population along the marginal stretch up to 600 m below the outfall is found to be significantly affected due to more adverse physical and chemical conditions in comparison to the winter (November-February). A noticeable decrease in plankton species, particularly zooplankton, was recorded up to a stretch of 100 m below the outfall during summer which might be due to presence of zinc around the outfall. While 24 h and 96 h LC50 values were found to be 41 and 43% by volume, respectively, for shrimps (Macrobrachium sp.), 95% confidence limits were found to lie between 28.2 and 59.4. Based on the bioassay result, dilution (1:98) available during May near the outfall area and 0.01 as the application factor, it is suggested that the existing flow of the Hooghly at this point needs 1.4 times dilution or the volume of the effluent has to be reduced or proper treatment of the waste water, i.e. recovery of zinc and reduction of free mineral acid, are essential for the safe survival, growth and reproduction of shrimp which migrate towards more saline zones for breeding.

ACUTE TOXICITY OF ABATE TO *Lebistes reticulatus* AND *Tilapia galilaea*

by

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The acute toxicity of Abate to *Lebistes reticulatus* and *Tilapia galilaea* has been studied. The method used was a static test in glass aquaria. Since Abate is insoluble in water, stock solutions of it were made by dissolving known weights of it in acetone and ethanol respectively. Different volumes of the stock solutions were diluted with aquarium water to the required concentrations. Two sets of controls were employed. The first control had solvents of concentrations as those in the strongest solutions in aquarium water, but the second contained only aquarium water. Ten fish were put into each tank and the solutions were changed once daily.

The 96 h LC50 was 1.9 ppm for *Lebistes reticulatus* and 0.47 ppm for *Tilapia galilaea*. The plots of median mortality times against concentrations produced curves which were asymptotic about the LC50 values for the two species.

When more dilute stock solutions of the pesticide in ethanol were employed, an LC50 as low as 0.26 ppm was obtained for *Tilapia galilaea*. Similar results were not obtained with the larvicide dissolved in acetone. This was suggestive of synergism between ethanol and Abate for *Tilapia galilaea*. Further experimentation is required to confirm this aspect.

BENZOPYRENE HYDROXYLASE INDUCTION - MOLECULAR RESPONSE TO OIL POLLUTION

by

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and
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Induction of benzo(a)pyrene hydroxylase (BPH) activity occurred in *Blennius pavo*, a species with a restricted territorial range, as a response to exposure to a Diesel 2 oil. A response delay of 14 days was found at a concentration of 170 ppm and of 3 days when water was saturated with Diesel 2 oil. A benthic chordate, *Microcosmus sulcatus*, showed no increase in BPH activity when exposed to these concentrations even after 30 days of exposure. After the fish were transferred to clean water, elevated BPH activity was maintained at a high level for at least a month.

Field observations revealed a great variation in the BPH activity from fish caught at different sites. Fish from oil contaminated sites had elevated levels of BPH activity.

Sardine schools caught at different sites had different, low, levels of BPH activities. However, specimens from the same school had similar levels of enzyme activity.

It seems that measurement of BPH activity in the liver of non-migrant fish could serve as a useful parameter for the evaluation of recent or long-term oil pollution at a given site.

BIOLOGICAL EVALUATION OF THERMAL EFFECTS ON SOME MARINE ORGANISMS OF THE GULF OF THAILAND

by

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Due to limited knowledge of thermal effects on marine organisms of the tropical region and the plan to construct a number of nuclear power plants in the South East Asia region, nine research activities are underway at the Department of Marine Science, Chulalongkorn University, in order to assess various aspects of thermal effects on marine organisms of the Gulf of Thailand. Up to now, the obtainable data reveal the following conclusions:

The avoidance temperature, the critical thermal maximum and the upper incipient lethal temperature were affected by the acclimation temperature. Increased acclimation temperature resulted in an increase in the mean avoidance temperature, the critical thermal maximum, and the upper incipient lethal temperature. The mean incipient lethal temperature of 14 marine species acclimated at ambient sea-water temperature is 36.8°C. Fish of five tropical marine species preferred temperatures that were very near to or the same as the acclimation temperature.

The toxicity of mercury as mercuric chloride to shrimp (Metapenaeus monoceros) increased as the water temperature increased. The temperatures that were lower and higher than ambient seawater temperature (23°C and 33°C) could decrease the predation rate of Lates calcarifer and Epinephelus tauvania. The technical problem of this research programme is the availability of test organisms and a limited number of scientific literature on thermal effects on marine organisms in the tropical region.

COPPER MINING WASTES AND THEIR INFLUENCE ON FISHERY RESOURCES

by

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An evaluation was made of the pollution by copper effluents discharged into the rivers of the Valparaíso-Aconcagua region and the concentration found was compared with that experimentally determined in bioassay tests carried out with common trout (Salmo trutta) for a 24 h period.

The most polluted water courses are, in the north, the Pedernal River, with a concentration of 0.1 ppm Cu in sites close to the washing outfall; in the central region, the Guayacan and Los Angeles Rivers, with a concentration of 0.9 and 0.4 ppm respectively, and, finally, in the southern sector, the Aconcagua River, with a concentration of 0.4 ppm.

The bioassays determined that, in water which contains 250 ppm $CaCO_3$, the 24 h LC50 is 0.26, with a confidence limit between 0.157 and 0.427 ppm.

This degree of acceptability could be attributed to the additive effects of water hardness and acid discharges from tanneries and metallurgic plants, effects which can complex Cu, thus decreasing its toxicity.

SOME ECOLOGICAL CONSIDERATIONS OF THE HOOGHLY ESTUARY POLLUTED BY DOMESTIC AND INDUSTRIAL WASTES UNDER TROPICAL ENVIRONMENT

by

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The impact of 77 and 175 mgd of liquid waste disposals respectively from industries, estates and municipalities in the Hooghly estuary flowing through Calcutta, was investigated during 1967-71 in a stretch of 92 km by a bio-ecological study. The study reveals that, during the summer months (March to June) conditions in the estuary, because of low water flow, become unfavourable for aquatic life by the constant addition of effluents. The plankton population in the outfall area is significantly affected and, in the main body of the estuary, is poor during summer. The density of benthic organisms in general is low and around the outfall area they are completely absent. The LC50 determined for Daphnia similis gives an indication of dilution in the estuary necessary for their healthy survival and growth. This shows that, during very low water level, there is no possibility of enough supply of fresh water either from upstream or from rains. The simultaneous discharge of effluents from all industries can undoubtedly bring the dilution ratio to a critical level which is rather similar to the one observed with Daphnia similis and such environment may be harmful to micro-organisms under prolonged exposure. Such waters may cause additional stress to test animals, particularly micro-organisms, when used as dilution water. Application factors with such water may differ in different seasons.

BIOASSAY PROGRAMME AND RESEARCH IN THE POLLUTION LABORATORY OF THE INSTITUTE FOR FISHERIES INVESTIGATIONS

by

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The Pollution Laboratory of the Institute for Fisheries Investigations, National Fisheries Office, Ministry of Agriculture, is carrying out a series of investigations on the pollution, arising from a variety of sources, of Lake Maracaibo. These investigations are:

1. Bioassay studies with effluents from the "El Tablazo" petrochemical complex, situated in the Maracaibo estuary, using species of commercial interest. These species are: Mullet (Mugil lisa) white shrimp (Penaeus schmitti) and snook (Centropomus undecimalis). Research has been designed to obtain knowledge of the Median Tolerance Limit with petrochemical effluents on the species mentioned with ammonia, phenol, high temperature and low concentration of dissolved oxygen. The TLms obtained were: 5.3, 5.5 and 3.0 mg/l for ammonia (as nitrogen); 37.4, 35.5 and 33.5°C for temperature; 3.5, 14.2 and 9.0 mg/l for phenol and 1.6, 1.3 and 1 mg/l for dissolved oxygen, for white shrimp (Penaeus schmitti), snook (Centropomus undecimalis) and mullet (Mugil lisa) respectively. The bioassays were performed for 24 hours in static system.

2. Temperature effects on the TLm of ammonia related to species already mentioned with concentrations of ammonia from 1 to 10 mg/l and temperatures from 28 to 38°C.

3. Bioassays for 96 hours with the blue crab (Callinectes sapidus) in order to establish the LC50 when submitted to the phosphorylate pesticide Parathion. the LC50 values obtained were: 0.0056 ppm for 96 hours, 0.0070 for 72 hours, 0.015 for 48 hours and 0.026 ppm for 24 hours. The bioassays were undertaken with concentrations between 0.002 and 0.040 ppm in a static system, with continuous aeration and gas chromatographic analysis to verify the concentrations.

BIOLOGICAL EFFECTS OF DOMESTIC AND INDUSTRIAL WASTES ON NATURAL ECOSYSTEMS IN URUGUAY*

by

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A comparative study has been carried out in Carrasco, Pantanoso, de Mendoza and Miguelete brooks, in the department of Montevideo, on the abiotic physico-chemical factors of the sediments and biotic components, plankton, bottom biota and fish in relation to pollution and its effects on the aquatic communities. The ecosystem of Cañada Tropa Vieja, an almost unpolluted stream, has been used as a reference.

The research team verified chronic pollution of multiple origin, biological, mechanical and thermal, and its widespread occurrence.

The results of the ecological survey showed that 55% of the length of the streams are severely damaged - an apparently low percentage - due to the fact that the majority of the industries are situated on their middle and lower courses.

This situation reduces the capacity of self-purification of the streams.

25% of the length of the rivers show less serious pollution and only 20% of the stream courses have water quality favourable to the development of aquatic life.

In all the streams, however, erosion symptoms are evident during certain periods of the year, due to the speed of the water, high enough to eliminate sedimentation processes.

Comparing the situation mentioned above with that observed at Cañada Tropa Vieja, we detected, in the latter, communities with higher diversity index of species represented and larger numbers of individuals of the taxa.

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*Not presented as meeting document

Annex VII

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Manual of Methods in Aquatic Environment Research. Part 4.
FAO Fisheries Technical Paper No. 164

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